

Purpose: Osteoarthritis (OA) is a chronic low-grade inflammatory disease for which the diagnosis currently relies on clinical and radiological means whereas sensitive serum biomarkers specific for an early stage of the disease are lacking. The aim of the study was to identify specific molecular alterations of articular cartilage in an in vitro inflammation model. The pro-inflammatory cytokine interleukin (IL)-1 β was used to induce degradation of articular cartilage explants cultured in vitro with the purpose of mimicking OA cartilage in early and later developmental stages. The specific objective was to longitudinally characterize and quantify the release of cartilage matrix components to the media by using a quantitative proteomic approach.

Methods: Full thickness equine articular cartilage explants were harvested from the weight bearing part of the distal metacarpal bone III in the metacarpal-phalangeal joint of three horses. The explants were cultured in the presence or absence of interleukin IL-1 β in-vitro and cell media was changed and sampled at day 3, 6, 9, 12, 15, 18 and 22. Proteins were isolated from the harvested media and following tryptic digestion the peptides were labeled with isobaric tandem mass tags (TMT) and analyzed by liquid chromatography-mass spectrometry (LC-MS/MS). The TMT reporter ion intensities in MS/MS spectra were divided by reference reporter ion intensities, giving a relative amount for each quantified peptide. The relative amount of released components in media from IL-1 β stimulated and unstimulated explants were compared over time.

Results: IL-1 β stimulation resulted in an abundance of proteins related to the extra cellular matrix and the proteins were released at different time points during culturing time. Release of several cartilage matrix components e.g. aggrecan, cartilage oligomeric matrix protein (COMP), chondroadherin, thrombospondin-1 and proteoglycan 4 displayed high amounts at days 3 and 6, similar to the early stage of the disease process of OA in vivo. Collagen type XII release was identified at day 9 and 12 and the release of collagen type VI increased continually from day 18–22. Several small leucine-rich proteoglycans (SLRP) such as biglycan and lumican had the highest release at day 22, together with a high amount of collagen type II in IL-1 β stimulated media. The unstimulated media showed signs of new synthesis of collagen type II, by the presence of high amount of procollagen type II C-propeptide and C endopeptidase enhancers, and this collagen synthesis was not at all prominent in the IL-1 β stimulated media.

Conclusions: This inflammatory in vitro model display structural changes seen at distinct developmental stages of OA in vivo. Novel findings presented are the release, at different time points after IL-1 β stimulation, of cartilage components in a specific time pattern in the order as follows; COMP, collagen type XII, collagen type VI, SLRPs and finally collagen type II, indicating a degradation of the collagen network in a distinct pattern, without evidence for new synthesis of collagen type II. The results from the unstimulated explants indicate repair capacity with an increase of collagen synthesis. The identification of early biochemical changes in cartilage would be of great importance in both finding disease modifying pharmaceutical compounds as well as in the development of biomarkers for early OA.

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IMMUNOHISTOCHEMICAL LOCALIZATION OF NOTCH SIGNALING COMPONENTS, MATRIX COMPONENTS AND STRO-1 IN PRENATAL, POSTNATAL AND OSTEOARTHRITIC EQUINE CARTILAGE

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Purpose: The growth plate and articular cartilage of developing synovial joints include specific zones of chondrocytes with different phenotypes. The chondrocytes in the growth plate change from resting to proliferative and hypertrophic phenotypes. Phenotypical changes also occur in the chondrocytes of the articular cartilage during the progression of osteoarthritis (OA). The expression of proteins on the chondrocytes as well as in the surrounding matrix affects the phenotype and can be used to characterize cells of specific phenotypes. The aim of the study was to localize components with potential effects on the phenotype such as the Notch signaling components including the receptor Notch1, the ligand Delta4 and the target gene Hes1. Additionally the stem cell indicating marker Stro-1 and matrix

molecules were localized to further characterize cells and matrix in developing prenatal and postnatal equine cartilage as well as in OA cartilage.

Methods: Immature equine osteochondral tissues, representing different developmental stages of the articular and growth cartilage of the distal radius were collected post mortem from two fetuses (6.5 and 10 months gestational age) and from two young horses (2-day-old and 1-year-old). Articular cartilage from fetlock joints of adult horses was collected and articular cartilage with superficial chondronecrosis, fibrillation and adjacent cluster formations, compatible with OA cartilage was identified by histology and included in the study. The localization of Notch signaling components, Stro-1 and the matrix molecules cartilage oligomeric matrix protein (COMP), fibromodulin, chondroadherin and matrilin-1 was examined by immunohistochemistry.

Results: Notch1, Delta4 and Hes1 indicating active Notch signaling was localized in hypertrophic cells in growth plates of the distal radius in all horses, as well as in resting cells of prenatal horses and in proliferative cells of the youngest fetus. The same proteins were present in all zones of the radial articular cartilage of the postnatal horses, whereas only a few cells were stained in the youngest fetus.

Stro-1 was present in the resting zone of the youngest fetus, with weak immunolabeling in the resting zone of the 10-month-old fetus and only detected as weak immunolabeling in the proliferative zone of the 2-day-old horse and not detected in the growth plate of the 1-year-old horse. In articular cartilage the chondrocytes stained positive for Stro-1 in all zones of the youngest fetus, whereas the staining became restricted to the superficial and middle zones in the postnatal horses. Most matrix molecules gradually changed in location with increasing age from interterritorial to pericellular location in the growth plates. The most prominent changes were observed in the hypertrophic zone where COMP, fibromodulin and chondroadherin were localized interterritorially in the prenatal horses but shifted to a pericellular location in the postnatal horses. Matrilin-1 was present in the hypertrophic zone of the prenatal horses but absent in the postnatal horses. In the articular cartilage the matrix molecules were present in all zones with an age related shift from interterritorial to pericellular location in all zones but the superficial zone, which displayed interterritorial staining in all horses. The Notch1 receptor and the Delta4 ligand were detected in all zones of OA cartilage with the strongest staining in the superficial zone and in cell clusters. Intense Stro-1 and weak Hes1 immunolabeling was detected in the superficial zone and in cell clusters. All matrix components were present in OA cartilage.

Conclusions: This study demonstrates presence of Notch signaling components in equine cartilage of different developmental stages which support previous findings of a role for Notch signaling in endochondral ossification and differentiation of chondrocytes. The studied matrix molecules known to be involved in the formation and organization of the collagen network showed age related differences in localization of the molecules, which may indicate increased mechanical load or altered expression related to different developmental stages. The weak staining of Hes1 in OA cartilage suggests low activity of the Notch1 signaling, and the strong staining of Stro-1 in the superficial zone and OA cell clusters indicates stem cell like characteristics of the chondrocytes. This is the first study demonstrating the localization of Notch signaling components, Stro-1 and matrix components in equine growth cartilage of different developmental stages and OA cartilage.

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OSTEOCLASTS REDUCE THE ACTIVATION OF WNT CANONICAL PATHWAY IN CHONDROCYTES

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Purpose: Osteoclastogenesis is enhanced in early osteoarthritis (OA). We have previously demonstrated that cartilage degradation is reduced in vivo when osteoclastogenesis is inhibited in murine models with high bone resorption. Wnt activity, known to regulate the bone and chondrocyte cell function, might contribute to the mechanisms that promote cartilage breakdown. Our purpose was to evaluate whether osteoclast-secreting molecules affect directly the metabolism of chondrocyte and the contribution of Wnt pathway.

Methods: Osteoclasts were obtained after differentiation of RAW cells cultured with RANKL. Primary murine chondrocyte (Ch) were then cultured in the presence of osteoclast conditioned medium (Oc-CM) for 48h. The gene and protein expressions of catabolism and anabolism